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INVITED RESEARCH HIGHLIGHT

Prostate Cancer

Long noncoding RNA-mediated activation of androgen receptor in prostate cancer

Gyorgy Petrovics, Shiv Srivastava

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Remarkable progress has been made in molecular characterization of prostate cancer (PCa) with continued innovations in high throughput technologies evaluating human cancer.[1-3] Since the completion of the Human Genome Project it has been estimated that only about 1.5%–2% of our genome codes for proteins. Various genome-wide approaches, e.g. the ENCODE project, revealed that a much larger percent of the genome is transcribed as non-protein coding (nc) RNA, including long noncoding (lnc) RNA (over 200 bps long). Although the biological roles of lncRNA (the 'dark matter of the genome') are not nearly as well-understood as the protein coding mRNAs, it is increasingly clear that they play important roles in almost every aspects of biology, including cancer biology.[4,5] This is exemplified by recent genome-wide association studies revealing that over 80% of cancer-associated single nucleotide polymorphisms (SNPs) are in noncoding regions of the genome.

Lnc RNAs can function through a variety of mechanisms, including chromatin remodeling (Xist, Hotair, Anril), transcriptional co-activation/repression (H19, lincRNA-p21, SRA), posttranscriptional modifications (MALAT1), protein inhibition (TERRA) and decoy elements (PTENP1).[4,5] *SRA* has been identified as a steroid receptor coactivator lncRNA.[6] However, the function and mechanism of most lnc RNAs remain unclear.

Surprisingly enough, early discoveries using differential display technologies described two lncRNAs, *DD3/PCA3*[7] and *PCGEM1*,[8] which not only exhibited high prostate tissue specificity but also showed prostate tumor associated overexpression. Recent evaluations of PCa transcriptome have described several lncRNAs, including *PCAT-1*, a transcriptional repressor and target of Polycomb Repressive Complex 2, implicated in PCa progression[9] and *PRNCR1*, a PCa susceptibility associated lncRNA.[10] Interestingly, both *PCAT-1* and *PRNCR1* reside in the 8q24 PCa susceptibility locus, less than a Mb from the *CMYC* locus which is often amplified in PCa. While functions of many of these lncRNAs remain to better understood in PCa biology, overexpression of *PCA3* in virtually all PCas has led to a recently FDA approved diagnostic test.[11,12]

The focus of this Nature report[13] is on two PCa-associated lncRNAs: P*CGEM1* and *PRNCR1*. They cooperate in regulating the function of the male hormone receptor, the androgen receptor (AR), which plays central role in PCa onset and progression. AR pathway is activated in advanced CaPs including castration-resistant prostate cancer (CRPC). *PCGEM1*, a prostate specific androgen regulated gene is expressed in approximately half of all PCas with significantly increased association in tumors of African American patients. *PCGEM1* also exhibits oncogenic activity in cancer cell biology experiments.[14,15] *PRNCR1* is transcribed from the 'gene desert' region of chromosome 8q24, strongly associated with susceptibility to PCa. It was described as a 13 kb intron-less lncRNA that affects transactivation activity of AR.[10]

The Yang *et al.*[13] Nature report connects the AR-regulated gene activation program with these two PCa-associated lncRNAs, *PCGEM1* and *PRNCR1*. In a series of experiments physical association between these PCa-associated lncRNAs and AR was established both in human prostate tumor tissue and in LNCaP cell line model. Hormonal induction of these associations was revealed by DHT treatment of LNCaP cells. Of note, previous studies have shown that *PCGEM1* itself can be induced by androgen,[8] which may further cooperate with AR activation especially when it is overexpressed in PCa. Antisense oligonucleotide based knockdown of *PRNCR1* abolished both its own interaction with AR, and the association of *PCGEM1* with AR. However, antisense oligonucleotide targeting of *PCGEM1* abolished only the *PCGEM1*-AR association, suggesting for a *PRNCR1* dependent recruitment of *PCGEM1* to AR. *In vitro* binding studies mapped the *PRNCR1* binding site to AR 549–623 region, and the *PCGEM1* binding site to the N-terminal region of AR. The lncRNA-bound AR had specific posttranslational modifications: acetylation was required for association with *PRNCR1* and methylation for the *PCGEM1* binding. These promising novel observations will lead to further refinement of these complex interactions.

Chromatin isolation by RNA purification (ChIRP) revealed over 2000 *PCGEM1* occupancy sites in the genome, about 80% of them colocalize with AR-bound sites. Global run-on sequencing (GRO-seq) revealed that knockdown of either lncRNAs by antisense oligonucleotide decreased AR target gene expression (about 600 genes). Similarly, shRNA against either *PCGEM1* or *PRNCR1* reduced the DHT-induced activation of AR targets without affecting AR expression levels. Significantly, the truncated AR-V7 (75 kDa) splice variant, which can activate AR-regulated genes without ligand (hormone), associated with both lncRNAs. Knockdown of either *PCGEM1* or *PRNCR1* inhibited AR-regulated gene activation by AR-V7.

Finally, the biological roles of these lncRNAs were investigated in stable cell lines

Center for Prostate Disease Research, Department of Surgery, Uniformed Services University of the Health Sciences, Maryland, USA.
Correspondence: Dr. G Petrovics (gpetrovics@cpdr.org)

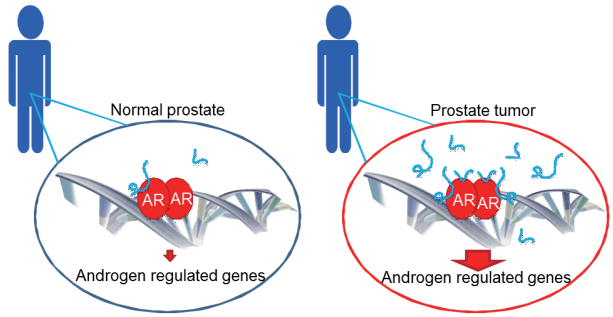

Figure 1: Schematic model illustrating AR (red dimer on DNA) activation by overexpressed lncRNAs *PCGEM1* and *PRNCR1* (blue ribbons) in prostate tumors. The increased thickness of the red arrow represents elevated mRNA expression of AR-regulated genes. AR: androgen receptor.

of CWR22Rv1 harboring dox-induced shRNA against *PCGEM1* or *PRNCR1*. In addition to reduced expression of canonical AR target genes a reduction of cell growth and inhibition of tumor growth in a xenograft model (CRPC) was demonstrated.

Taken together, novel findings from this report, along with previous studies of *PCGEM1*, AR and *PRNCR1*, reveal biological importance of prostate associated lncRNAs (*PCGEM1* and *PRNCR1*) in full length and truncated AR-dependent gene activation in PCa (**Figure 1**). As *PCGEM1* and *PRNCR1* strongly enhance AR activity in PCa, they may be explored as potential new therapeutic targets in CRPC.

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